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Dietary Nitrosodimethylamine and the Risk of Lung Cancer: A Case-Control Study from Uruguay[1]

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Abstract

Evidence from animal studies indicates that various *N*-nitroso compounds are carcinogenic. We investigated whether consumption of nitrosodimethylamine (NDMA) and foods and beverages containing NDMA are carcinogenic for the lung. In a hospital-based case-control study in Uruguay, dietary intake of NDMA and its food sources was measured in 320 cases of lung cancer and 320 controls afflicted with diseases not related with tobacco use and diet. After adjusting for tobacco smoking and total energy intake, NDMA displayed a significant dose-response pattern, with a 3-fold increase in risk for the higher category of intake. The risks were slightly more elevated for adenocarcinoma of the lung. Also, salted meat consumption and beer intake were associated with an increased risk of lung cancer.

Introduction

In 1960, Zak *et al.* (1) reported the occurrence of lung carcinomas in rats treated with NDMA[3]. This observation was consistently replicated in other experiments and, as a result, NDMA has been considered carcinogenic in all animal species tested. In fact, NDMA induces benign and malignant tumors after its administration by various routes (including ingestion) in various organs - mainly the kidney, liver, and respiratory tract (2). Because NDMA is present in several foods and beverages, its role in human carcinogenesis has been considered measurable, and several authors (3–5) have reported an increased risk of stomach, esophageal, pharyngeal, laryngeal, and lung cancer associated with a high intake of NDMA.

Because the Uruguay population has a high consumption of cured meats and other sources of NDMA, a case-control study was designed to evaluate the possible association between dietary NDMA and lung cancer in this country.

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[3] The abbreviations used are: NDMA, nitrosodimethylamine; OR, odds ratio; GLIM, generalized linear interactive modeling; CI, confidence interval.

Patients and Methods

During the study period May 1994–December 1995, all incident cases of lung cancer admitted for treatment in the seven major hospitals of Montevideo were considered as eligible for the present study. In total, 320 lung cancer cases were identified in the participant hospitals (307 men and 13 women), with an average age of 63.1 years. Squamous cell cancer was the most frequent cell type (153 cases, 47.8%), followed by adenocarcinoma (65 cases, 20.3%), small cell (36 cases, 11.3%), and large cell anaplastic type (8 cases, 2.5%). Ten cases (3.1%) were diagnosed as carcinoma not otherwise specified, and 48 cases (15.0%) were diagnosed solely on clinical, radiological, and endoscopic grounds (Table 1).

One control, frequency matched on age (5-year age groups), sex, and residence (Montevideo, other counties), was selected for each case from the admissions register of the same hospital in which the case was diagnosed. The controls excluded subjects hospitalized for diseases related with tobacco use. The most frequent conditions were fractures (36.2%), eye disorders (21.6%), abdominal hernia (15.1%), trauma (8.8%), and appendicitis (5.3%; Table 2).

Interviews were carried out by trained interviewers while both cases and controls were still hospitalized. Because of good cooperation, all the subjects identified and qualifying for the study agreed to be interviewed. The questionnaire included a section on demographics, a complete tobacco history, data on consumption of alcoholic and nonalcoholic beverages, and a food section.

Dietary data were collected by a food-frequency questionnaire, which estimates the usual diet during the 5 years preceding the onset of illness. Queries on 70 food items, using the usual portion size, were ascertained. These food items allowed the calculation of total energy intake. Concerning NDMA, the main sources included in the questionnaire were bacon, sausage, mortadella, salami, saucisson, frankfurter, ham, salted meat, fish, poultry, cheese, beer, and hard liquor.

Nutrient intake was estimated on the basis of a local conversion table (6). Values for nitrate, nitrite, and NDMA were obtained from several publications that included 26 of the most frequently consumed food groups, particularly those considered to be the main sources of these nitroso compounds (7–11). Nutrient intake was categorized in four levels, using as cutpoints the 25, 50, and 75 quartiles of the combined sample of cases and controls. Nutrients were adjusted for total energy after using the residual analysis method of Willett and Stampfer (12).

Estimation of the ORs was based on unconditional logistic regression following the methods described by Breslow and Day (13). The possible confounding effect of other variables was controlled for in multiple logistic regression models. The lowest level consumption of foods and nutrients was used as the reference category in the estimation of the OR. Ninety-five percent confidence bounds were calculated. Categorical vari-

Table 1 Distribution of cases by cell type

Cell type	No.	%
Squamous cell	153	47.8
Small cell	36	11.3
Adenocarcinoma	65	20.3
Large cell	8	2.5
Carcinoma NOS[a]	10	3.1
Clinical diagnosis	48	15.0
	320	100.0

[a] NOS, not otherwise specified.

Table 2 Distribution of controls by disease category (ICD-9 classification)

ICD-9	Category	No.	%
800–839	Fractures	116	36.2
360–379	Eye disorders	69	21.6
550–553	Abdominal hernia	48	15.1
830–959	Trauma	28	8.8
540	Appendicitis	17	5.3
680–709	Skin diseases	12	3.7
710–739	Osteoarticular diseases	12	3.7
454	Varicose veins	10	3.1
011–122	Infectious diseases	8	2.5
		320	100.0

ables were used for calculation of the χ^2 test for trend over four quartiles. All calculations were performed using the GLIM software (14).

Results

The distribution of cases and controls by sociodemographic variables is shown in Table 3. As expected, grouped age, sex, and residence were identical among both series. Also, urban/rural status, education, and monthly income were similar in cases and controls.

ORs for tobacco variables are shown in Table 4. All smokers were associated with an increased risk of 8.7, whereas current smokers displayed an OR of 9.1 (95% CI, 5.2–15.9). Pack-years of tobacco smoking were strongly associated with lung cancer risk (the OR for the higher exposure category was 16.1; 95% CI, 8.6–30.4). Smokers of black tobacco were at increased risk of lung cancer (OR, 11.2) compared to smokers of blond (flue-cured) tobacco (OR, 4.7). Also, smokers of hand-rolled cigarettes were associated with a strong risk (OR, 10.0) compared to smokers of manufactured (commercial) cigarettes (OR, 5.0). Finally, smokers of plain cigarettes showed an increased risk of 10.1, compared with a risk of 7.4 for smokers of filter cigarettes.

ORs for foods and beverages containing NDMA are shown in Table 5. Increased risks were observed for salami, poultry, salted meat, beer, and hard liquor. ORs for NDMA are shown in Table 6. For the entire series, NDMA intake was associated with a monotonic gradient of increased risks, with a highly significant test for linear trend ($P < 0.001$). A 3-fold increase in risk was observed for the highest level of intake. Both squamous cell and adenocarcinoma types were associated with a strong dose-response pattern, and the highest category of intake showed an increased risk of 4.6 for adenocarcinoma of the lung. Further adjustment for alcohol intake left the estimates unchanged (results not shown).

Table 3 Distribution of cases and controls by sociodemographic variables

Variable	Cases (%)	Controls (%)
Age (yrs)		
30–39	5 (1.6)	5 (1.6)
40–49	36 (11.3)	36 (11.3)
50–59	67 (20.9)	67 (20.9)
60–69	132 (41.3)	132 (41.3)
70–79	75 (23.4)	75 (23.4)
80–89	5 (1.6)	5 (1.6)
Sex		
Male	307 (95.9)	307 (95.9)
Female	13 (4.1)	13 (4.1)
Residence		
Montevideo	152 (47.5)	152 (47.5)
Other counties	168 (52.5)	168 (52.5)
Urban/rural status		
Urban	241 (75.3)	237 (74.1)
Rural	79 (24.7)	83 (25.9)
Education (yrs)		
0–3	137 (42.8)	140 (43.8)
4–6	147 (45.9)	136 (42.5)
7+	36 (11.3)	44 (13.8)
Income (dollars)		
≤127	143 (44.7)	129 (40.3)
≥128	135 (42.2)	133 (41.6)
Unknown	42 (13.1)	58 (18.1)
No. of patients	320 (100)	320 (100)

Table 4 ORs and 95% CIs for tobacco variables[a]

Variable	Category	Cases/controls	OR	95% CI
Smoking status	Nonsmokers	20/108	1.0	–
	Ex-smokers	84/61	8.1	4.5–14.7
	Current smokers	216/151	9.1	5.2–15.9
	All smokers	300/212	8.7	5.1–14.9
Pack-years	Nonsmokers	20/108	1.0	–
	1–33	37/92	1.9	1.0–3.8
	34–54	73/56	6.5	3.5–12.1
	55–84	95/33	14.5	7.7–27.2
	85+	95/31	16.1	8.6–30.4
Type of tobacco	Blond	80/99	4.7	2.6–8.6
	Black	220/113	11.2	6.4–19.3
Hand-rolling	Manufactured	68/79	5.0	2.6–9.4
	Hand-rolled	232/133	10.0	5.8–17.2
Filter use	Filter	158/128	7.4	4.2–13.2
	Plain	142/84	10.1	5.7–17.8

[a] ORs adjusted for age, sex, residence, urban/rural status, education, family history of lung cancer, and body mass index.

When NDMA was stratified by type of tobacco smoked and hand-rolling status, a stronger effect was observed among smokers of blond tobacco (OR, 6.6; 95% CI, 2.4–18.1) than among smokers of black tobacco. When this effect modification was statistically tested for interaction, the results were nonsignificant ($\chi^2 = 0.14$; $P = 0.71$). Also, smokers of hand-rolled cigarettes were associated with a stronger risk (OR, 3.5; 95% CI, 1.7–6.9) than that shown by smokers of commercial cigarettes (OR, 1.7; 95% CI, 0.6–5.1). The interaction was not significant ($\chi^2 = 1.38$; $P = 0.24$). Finally, stratification by smoking status showed similar ORs for nonsmokers, former smokers, and current smokers (Table 7).

Table 5 ORs and 95% CIs for foods and beverages containing NDMA[a]

Food/beverage	NDMA concentration[b]	OR	95% CI
Fish[c]	0.20	1.24	0.87–1.78
Poultry[c]	0.10	1.40	0.97–2.02
Bacon[c]	0.27	0.81	0.57–1.16
Sausage[c]	0.30	0.96	0.66–1.37
Mortadella[c]	0.40	1.01	0.71–1.45
Salami[c]	1.91	1.31	0.91–1.89
Saucisson[c]	1.91	1.09	0.76–1.55
Frankfurter[c]	0.20	0.93	0.65–1.33
Ham[c]	0.40	1.06	0.74–1.52
Salted meat[c]	2.30	1.56	1.01–2.42
Cheese[c]	0.10	1.01	0.70–1.48
Beer[d]	0.44	1.89	0.96–3.73
Hard liquor[d]	0.19	1.43	0.98–2.09

[a] ORs adjusted for age (continuous), sex, residence, urban/rural status, education, family history of lung cancer, body mass index, pack-years (continuous), and total energy.
[b] NDMA concentrations in μg/kg.
[c] ORs for consumption one or more times/week. Reference category: consumers of less than one time/week.
[d] ORs for drinkers. Reference category: never drinkers of beer and hard liquor.

Table 6 ORs and 95% CIs for NDMA by cell type[a]

Cell type		NDMA ≤0.13	NDMA 0.14–0.18	NDMA 0.19–0.26	NDMA ≥0.27
All types	Cases	67/92	66/94	82/79	105/55
	OR1	1.0	0.96	1.42	2.62
	OR2	1.0	0.88	1.77	3.14
	95% CI	–	0.53–1.48	1.06–2.96	1.86–5.29
Squamous cell	Cases	26/92	32/94	47/79	48/55
	OR1	1.0	1.20	2.11	3.09
	OR2	1.0	1.08	2.34	3.11
	95% CI	–	0.56–2.10	1.24–4.42	1.62–5.95
Small cell	Cases	10/92	9/94	5/79	12/55
	OR1	1.0	0.88	0.58	2.01
	OR2	1.0	0.78	0.93	2.41
	95% CI	–	0.26–2.31	0.27–3.20	0.82–7.13
Adenocarcinoma	Cases	13/92	11/94	19/79	22/55
	OR1	1.0	0.82	1.70	2.83
	OR2	1.0	0.87	2.78	4.57
	95% CI	–	0.33–2.29	1.13–6.85	1.88–11.1

[a] ORs adjusted for age, sex, residence, urban/rural status, family history of lung cancer, body mass index, pack-years, and total energy intake. OR1, crude ORs; OR2, adjusted ORs.

Table 7 ORs for the effect of NDMA stratified by type of tobacco and hand-rolling[a]

NDMA	Type of tobacco: Blond	Type of tobacco: Black
≤0.13	1.0 –	1.0 –
0.14–0.18	1.24 (0.47–3.29)	0.64 (0.32–1.25)
0.19–0.26	2.70 (0.99–7.32)	1.61 (0.78–3.30)
≥0.27	6.63 (2.42–18.1)	2.16 (1.03–4.53)

NDMA	Hand-rolling: Manufactured	Hand-rolling: Rolled
≤0.13	1.0 –	1.0 –
0.14–0.18	0.86 (0.31–2.38)	0.79 (0.41–1.53)
0.19–0.26	1.19 (0.37–3.78)	2.00 (1.02–3.91)
≥0.27	1.68 (0.55–5.13)	3.46 (1.71–6.97)

NDMA	Smoking status: Nonsmokers	Smoking status: Former smokers	Smoking status: Current smokers
≤0.13	1.0 –	1.0 –	1.0 –
0.14–0.18	1.06 (0.14–7.74)	0.49 (0.17–1.38)	1.12 (0.62–2.03)
0.19–0.26	1.35 (0.28–6.45)	1.09 (0.39–3.05)	2.0 (1.07–3.82)
≥0.27	3.42 (0.76–15.4)	2.25 (0.77–6.58)	2.95 (1.53–5.64)

[a] ORs adjusted for age, sex, residence, urban/rural status, education, family history of lung cancer, body mass index, pack-years, and total energy intake. CIs among parentheses. For nonsmokers, the term for pack-years was deleted.

Discussion

The present study provides additional evidence of an independent effect of dietary NDMA in the risk of lung cancer. Our food-frequency questionnaire was comprehensive and included foods and beverages that allowed a rather precise estimation of NDMA intake in the diet. Furthermore, the estimates of NDMA were fully adjusted for potential confounders like body mass index, total energy intake, and pack-years of cigarette smoking. The inclusion of terms for β-carotene, total carotenoid intake, total fat, saturated fat, cholesterol, vitamin C, and total alcohol intake left the estimates for NDMA unchanged. In particular, we attempted to minimize residual confounding from tobacco use, including a term for pack-years as a continuous variable, terms for smoking status, and assigning a value of 0 pack-years for nonsmokers. Also, stratification by smoking status was not suggestive of residual confounding.

The relationship between nitrosamine intake and human cancer has been the subject of several case-control studies (3–5). According to them, NDMA intake is a risk factor for gastric, esophageal, laryngeal, and oropharyngeal cancer. Recently, Goodman *et al.* (5) reported an increased risk of lung cancer associated with NDMA intake. It has been suggested that *N*-nitroso compounds are more effective as carcinogens in animals when taken per os and given in small quantities over time. This situation is similar to that observed in a human lifetime (4).

Consumption of certain foods containing NDMA was associated with a higher risk of developing lung cancer in this study. This was particularly evident with the consumption of salted meat. This food item has been associated with an increased risk of oropharyngeal and laryngeal cancer in humans (15–18). Also, drinkers of beer were at increased risk of developing lung cancer (OR, 1.89; 95% CI, 0.9–3.7), replicating previous findings (19, 20).

In summary, NDMA intake was associated in this particular population with an increased risk of lung cancer. Also, salted meat and beer proved to be risk factors for this disease.

References

1. Zak, F. G., Holzner, J. H., Singer, E. J., and Popper, H. Renal and pulmonary tumors in rats fed dimethylnitrosamine. Cancer Res., *20:* 96–99, 1960.

2. IARC. IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans: Some *N*-Nitroso Compounds. Lyon, France: IARC, 1978.

3. Gonzalez, C. A., Riboli, E., Badosa, J., Batiste, E., Cardona, T., Pita, S., Sanz, J. M., Torrent, M., and Agudo, A. Nutritional factors and gastric cancer in Spain. Am. J. Epidemiol., *139:* 466–473, 1994.

4. Rogers, M. A. M., Vaughan, T. L., Davis, S., and Thomas, D. B. Consumption of nitrate, nitrite, and nitrosodimethylamine and the risk of upper aerodigestive tract cancer. Cancer Epidemiol., Biomarkers & Prev., *4:* 29–36, 1995.

5. Goodman, M. T., Hankin, J. H., Wilkens, L. R., and Kolonel, L. N. High-fat foods and the risk of lung cancer. Epidemiology (Cambridge, MA), *3:* 288–299, 1992.

6. Mazzei, M. E., and Puchulu, M. R. Table of Chemical Composition of Foods (in Spanish). Buenos Aires, Argentina: Cenexa, 1991.

7. Cornee, J., Lairon, D., Velema, J., Guyader, M., and Berthezene, P. An estimate of nitrate, nitrite, and *N*-nitrosodimethylamine concentrations in French food products or food groups. Sci. Aliments, *12:* 155–197, 1992.

8. Havery, D. C., Kline, D. A., Miletta, E. M., Joe, F. L., and Fazio, T. Survey of food products for volatile *N*-nitrosamines. J. Assoc. Off. Anal. Chem., *59:* 540–546, 1976.

9. Panalaks, T., Iyengar, J. R., and Sen, N. P. Nitrate, nitrite, and dimethylnitrosamine in cured meat products. J. Assoc. Off. Anal. Chem., *56:* 621–625, 1973.

10. Panalaks, T., Iyengar, J. R., Donaldson, B. A., Miles, W. F., and Sen, N. P. Further survey of cured meat products for volatile *N*-nitrosamines. J. Assoc. Off. Anal. Chem., *57:* 806–812, 1974.

11. Gough, T. A., Webb, K. S., and Coleman, R. F. Estimate of the volatile nitrosamine content of U.K. food. Nature (Lond.), *272:* 161–163, 1978.

12. Willett, W. C., and Stampfer, M. J. Total energy intake: implications for epidemiologic analyses. Am. J. Epidemiol., *124:* 17–27, 1986.

13. Breslow, N. E., and Day, N. E. Statistical Methods in Cancer Research. I. The Analysis of Case-Control Studies. IARC Scientific Publ. No. 32. Lyon, France: IARC, 1980.

14. Baker, R. J., and Nelder, J. A. The GLIM System: Release 3.77. Oxford, United Kingdom: Numerical Algorithms Group, 1985.

15. Zheng, W., Blot, W. J., Shu, X-O., Diamond, E. L., Gao, Y-T., Ji, B-T., and Fraumeni, J. F., Jr. Risk factors for oral and pharyngeal cancer in Shanghai, with emphasis on diet. Cancer Epidemiol., Biomarkers & Prev., *1:* 441–448, 1992.

16. De Stefani, E., Oreggia, F., Ronco, A., Fierro, L., and Rivero, S. Salted meat consumption as a risk factor for cancer of the oral cavity and pharynx: a case-control study from Uruguay. Cancer Epidemiol., Biomarkers & Prev., *3:* 381–385, 1994.

17. Zheng, W., Blot, W. J., Shu, X., Gao, Y., Ji, B., Ziegler, R. G., and Fraumeni, J. F., Jr. Diet and other risk factors for laryngeal cancer in Shanghai, China. Am. J. Epidemiol., *136:* 178–191, 1992.

18. De Stefani, E., Oreggia, F., Rivero, S., Ronco, A. and Fierro, L. Salted meat consumption and the risk of laryngeal cancer. Eur. J. Epidemiol., *11:* 177–180, 1995.

19. Potter, J. D., Sellers, T. A., and Folson, A. R. Beer and lung cancer in older women: the Iowa Women's Health Study. Am. J. Epidemiol., *132:* 784, 1990.

20. De Stefani, E., Correa, P., Fierro, L., Fontham, E. T. H., Chen, V., and Zavala, D. The effect of alcohol on the risk of lung cancer in Uruguay. Cancer Epidemiol., Biomarkers & Prev., *2:* 21–26, 1993.

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